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(54) Title: METHOD OF ESTABLISHING CULTURES OF HUMAN DENDRITIC CELLS AND USE THEREOF

(57) Abstract: A simple method for producing dendritic cells from peripheral blood monocytes is provided. The dendritic cells may be used as adjuvants for vaccines and immunotherapies. The mature dendritic cells also provide an effective means of producting novel T cell dependent antigens comprised of dendritic cell modified antigens useful as vaccines or for the treatment of disease.

TITLE OF THE INVENTION

Method of Establishing Cultures of Human Dendritic Cells and Use Thereof

Field of the invention

This invention relates to a method of establishing cultures of dendritic cells from peripheral blood monocytes. This invention also relates to vaccines, methods of immunizing animals and humans using the dendritic cells of the invention.

Background of the invention

Dendritic cells (DC), present in small numbers in most tissues (lymphoid and non-lymphoid), have been referred to as "nature's adjuvant". Dendritic cells play a crucial role in the inititation of T-cell dependent responses. DC bind and modify antigens such that the modified antigen when presented on the surface of DC can activate T-cells to participate in the immune response and the production of antibodies. Modifications of the antigen by DC would include fragmenting a protein into immunogenic peptides which can activate T-cells. DC are the most potent antigen-presenting cells (APC) due to their extreme capacity in stimulating naive T cells and maintaining adaptive immune responses (Steinman 1991 Annu. Rev. Immunol. 9, 271; Banchereau and Steinman 1998, Nature 360, 258). However, the use of these cells in producing a strong immune response and for identifying and extracting immunogenic peptides is hampered by the small numbers of these specialized antigen presenting cells in any given organ. In human blood, for example, about 0.1% of the white blood cells are dendritic cells and

methods to develop, in culture, a large number of dendritic cells have been difficult. DC originate in bone marrow and are known to develop along a myeloid or lymphoid lineage of differentiation based on phenotypes and culture conditions (Caux et al., 1992, Nature 360, 258; Sallusto and Lanzavecchia 1994, J. Exp. Med. 179, 1109; Zhou and Tedder 1996, Proc. Natl. Acad. Sci. USA 93, 2588; Saunders et al. 1996, J. Exp. Med. 184, 2185; Bykovskaja, et al., 1999, J. Leuk. Biol. 66, 659). The most effective method for producing DC from monocytes and CD34+ precursors of DC requires the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF) for differentiation, Interleukin 4 (IL-4) to suppress macrophage outgrowth, and a secondary signal such as tumor necrosis factor alpha (TNFα) or lipopolysaccharides (LPS) in ex vivo culture (Sallusto et al. 1994, supra; Chapuis et al., 1997, Eur. J. Immunol. 27, 431; Zhou et al., 1996, supra). This method is cumbersome and difficult to reproduce because of the requirement to use multiple activation and maturation factors. In addition, these methods are not known to duplicate a physiologically relevant pathway and therefore may introduce unwanted features such as toxicity, autoimmunity, immunosuppression and other unpredictable consequences when administered in treatment of a subject.

In an effort to produce larger numbers of DC which duplicate naturally produced DC, we examined the direct effect of different factors in supporting the maturation DC in ex vivo culture. Surprisingly, we found that the use of IL-15 alone was sufficient for differentiating CD14+ monocytes into DC.

Since its discovery in 1994 (Grabstein et al., 1994, Science 264, 965), most research on IL-15 has focused on the effects that this cytokine has on

lymphocytes, natural killer cells (NK) and natural killer T (NKT) cells because of its similarity with another T-cell cytokine IL-2. IL-2 and IL-15 share both the IL-2/IL-15β and IL-2R common γ chains (Lodolce, et al., 1998, Immunity 9, 669; Waldmann and Tagaya, 1999, Immunol. 17, 19; Armitage et al., 1995, Immunol. 154, 483), and so it was assumed that since IL-2 is primarily a T-lymphocyte growth factor, the activity of IL-15 must also be directed towards T cells. Furthermore, IL-15 was not considered to be involved in the induction of dendritic cells but was only known to have an anti-apoptotic effect that increased dendritic cell survival (Pirtskhalaishvili et al., 2000, Br. J. Cancer 83, 506-513). Therefore, the activation and maturation of monocytes following treatment with IL-15 is a new and unexpected observation.

SUMMARY OF THE INVENTION

Therefore, this invention provides a simple one-step method for producing a population of dendritic cell from CD14+ monocytes. The method comprises culturing CD14+ monocytes in the presence of IL-15 cytokine or a biologically active derivative of IL-15. A mature DC is generally characterized by up regulation of MHC class II, co-stimulatory molecules such as CD80 and CD86 and the ability to activate naive T cells. Therefore, in this study, the conversion of CD14+ monocytes to DC upon IL-15 treatment were characterized by measurements of phenotypic changes in cell morphology, expression of MHC class II and co-stimulatory molecules, expression of chemokines and the induction of antigen-specific responses from T cells.

The methods used to support this invention relied on purified CD14+ monocytes for the purpose of

confirming the target cell. Other monocytes or cell lineages may also be transformed to DC by the actions of IL-15 such as CD14-negative monocytes, natural killer T cells (NKT), certain long-term cultured cell lines, monocytic leukemia cells, and many other cells that express the IL-15 receptor. IL-15 alone was sufficient to cause the transformation of DC. However, the addition of other factors may be anticipated to induce additional, and in some cases, desirable, attributes in the resultant DC. Monocytes obtained from peripheral or cord blood are preferred because of their ease in processing.

This invention also provides dendritic cells in amounts which may be used therapeutically and which also may be used to prepare new therapeutic antigens. In addition, the dendritic cells prepared according to the method of this invention are also provided.

In another aspect, the present invention provides antigen-exposed dendritic cells prepared according to the method of the invention in which antigen-exposed dendritic cells have been exposed to antigen and express modified antigens for presentation to and activation of T cells.

The invention also provides novel antigens which are produced by exposing an antigen to cultures of dendritic cells prepared according to the method of the invention in which the antigen is modified by the dendritic cells to produce modified antigens which are immunogenic fragments of the unmodified or native antigen and which fragments activate T cells. The novel antigens may be used to immunize animals and humans to prevent or treat disease.

The invention also provides a method of preparing antigens from dendritic cells comprising providing dendritic cells prepared by the method of the

invention, contacting the cells with antigen for a period of time sufficient to allow the dendritic cell to phagocytose the antigen and process and present the antigen. The antigens processed by the dendritic cell may themselves be used alone or in combination with adjuvants included to evoke an immune response in an individual to the antigen.

In another embodiment, the present invention provides self-peptide antigens produced by pulsing the dendritic cells of the invention with a protein to which an individual has developed an immune response and extracting the relevant self-peptide or autoantigen. The autoantigen can be used in a method for treating an individual with an autoimmune disease by administering to the individual a therapeutically effective amount of self-peptides produced according to the method of the invention to induce tolerance to self-proteins. These methods and compositions can be used to treat autoimmune diseases selected from the group of juvenile diabetes, myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosis, ankylosing spondilitis, multiple sclerosis, and other diseases is also provided.

The invention also provides treatment for inflammatory diseases in which the pathogenesis involves exaggerated T cell mediated immune responses such as those present in atopic dermatitis and contact dermatitis, inflammatory bowel disease, graft vs. host disease in organ transplantation, and other diseases. IL-15 can be given to the patient for the purpose of activating dendritic cells at a critical time that will result in inactivation of autoimmune responses. Alternatively, dendritic cells can be activated by IL-15 in vitro, incubated with antigens, or poly-nucleic acids that encode such antigens, that are specifically

related to the autoimmune disease, and infused into the patient to diminish autoimmune inflammation.

In another aspect, the present invention relates to a method for providing an antigen to a host comprising exposing an antigen to a culture of dendritic cells prepared according to the present invention to produce antigen-exposed dendritic cells followed by inoculating the host with the antigen-exposed dendritic cells.

This invention also provides a method for activating T cells comprising the use of dendritic cells for capturing protein, viral, parasitic, and microbial antigens in an immunogenic form in situ and then presenting these antigens in a potent manner to T cells either in vitro or in situ.

The invention further provides a method for making antigenic peptides that are more specific to an individual's MHC molecules, thereby increasing the number of dendritic cells that harbor immunostimulatory peptides and therefore enhancing the level of specific immune response. Complex antigens, such as viruses, bacteria, parasites, can be cultured with IL-15 derived dendritic cells. Peptides that were processed by the dendritic cells can be eluted from the whole cells or from isolated MHC molecules and these processed antigens can be used to enhance the level of specific immunity.

Vaccines comprised of any of the antigens or antigen-exposed dendritic cells described above are also provided as are the methods of immunizing against diseases in humans or animals comprising administering any of the compositions of the invention. Compositions and methods for treating infectious diseases including mycobacteria, bacteria, parasites, and viruses are also related aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Culture with IL-15 causes CD14+ monocytes to acquire the characteristic morphology of mature dendritic cells in culture. Mononuclear cells were separated from blood by Ficoll-Hypaque density gradient centrifugation. CD14+ monocytes were positively selected from mononuclear cells using anti-CD14 mAb coated paramagnetic beads (Milteneyi Biotech. Inc., Auburn, CA) and then passed through an ironfiber column placed in a strong magnetic field (VarioMACS, Milteneyi Biotech., Inc.). CD14+ cells bound to the column were eluted. (A) CD14+ monocytes prior to culture; (B) CD14+ monocytes cultured for 6 days with GM-CSF and + IL-4, and followed by 24 h TNF-α stimulation; (C) CD14+ monocytes cultured for 7 days with IL-15 alone. Magnification 40x.

Figure 2. Kinetics of cell surface HLA-DR and CD86 expression. CD14+ monocytes were cultured with or without IL-15 and, harvested at different time points, and labeled with FITC-conjugated mAb for HLA-DR or CD86 antigens and then analyzed for cell surface expression of HLA-DR and CD86 by flow cytometry. (A), Kinetics of HLA-DR expression; (B), kinetics of CD86 expression. Open circles and triangles represent matched Ab isotype control, filled circles represent expression without cytokine treatment and; filled triangles represent expression in the presence of IL-15. Data are presented as the median value of fluorescence intensity.

Figure 3. Phenotypic Comparison of changes in cell surface receptors of CD14+ monocytes upon culture with IL-15 or the combination of GM-CSF, IL-4 and TNF-

α. Mature DC were generated from CD14+ monocytes either by treatment with IL-15 or by a conventional protocol using the combination of GM-CSF, IL-4 and TNF-α. DC were harvested after 7 days in culture and stained either with FITC-labeled or PE-labeled mAb and isotype matched control antibody. Cell surface antigen expression was measured by flow cytometry. (A) CD14+ cells prior to culture. (B) Comparison of mature DC generated by treatment with the combination of GM-CSF, IL-4 and TNF-α or IL-15 alone. Histograms represent thin lines for isotype-matched control antibody mAb, and thick lines for surface antigen.

Figure 4. Conversion of CD14+ monocytes to mature dendritic cells by IL-15 is independent of the GM-CSF, IL-4 and TNF-α-driven pathway. CD14+ monocytes were cultured with IL-15 in the presence of anti-GM-CSF antibody or control antibody (normal mouse serum, NMS). Cells were harvested at day 7, stained for cell surface HLA-DR (A) and CD86 (B) expression and measured by flow cytometry using FITC-labeled specific (thick line) or mAb and isotype matched control mAbs (thin line). Cell surface HLA-DR and CD86 expression were assessed by flow cytometry. Histogram (Fig. A and B) represents thin line for isotype matched control antibody and thick line for HLA-DR expression by CD14+ monocytes when cultured in the presence of IL-15; dotted and dashed lines represent HLA-DR expression when cultured with IL-15 in the presence of NMS or anti-GM-CSF antibody as indicated. Data presented in figures represent optimum dose of antibody concentration.

Figure 5. IL-15 induced mature dendritic cells stimulate a strong response from T cells obtained from an unrelated donor (allo response). DC from CD14+

monocytes were generated either by IL-15 or by a conventional protocol using a treatment with the combination of GM-CSF, IL-4 and TNF-α. Mature DC were harvested at day 7, γ-irradiated and used as stimulatory cells in the allogeneic MLR. T-cells were separated from peripheral blood by Ficoll-Hypaque and a discontinuous Percoll gradient as described in Materials and Methods. T cells ($2x10^5$) were cultured in a 96-well micro titer plate with $2x10^4$ and $2x10^5$ stimulatory cells. Proliferation was measured at day 6 after 10 to 12-h pulse with 1 μCi of [^{3}H]thymidine. Open and dark hatched bars represent proliferation of DC (without T cells); open and dark bars represent T-cell responses induced by DC that were cultured with GM-CSF/IL-4+TNF-α and IL-15, respectively. Results are from a representative experiment (total of four experiments).

Figure 6.Chemokines production by mature DC. Transcriptional activation of chemokine genes was measured by RT-PCR using cells prior to culture and CD14+ monocytes that were treated with either GM-CSF and IL-4 for 6 days followed by 24 h stimulation with TNFα or only with IL-15 for 7 days. Gels show the patterns of inflammatory and constitutive chemokine expression by monocytes prior to culture, DC generated by treatment with the combination of GM-CSF, IL-4 and TNF-α, or IL-15 treatment alone. A total of 30 PCR cycles were used. Amplification of β-actin from the same RNA samples was used a control.

DETAILED DESCRIPTION

The invention relates to a method for producing cultures of dendritic cells. The dendritic cells produced by the method of the inventon may be produced in amounts suitable for various immunological

interventions for the prevention and treatment of disease.

The starting material for the method of producing dendritic cells is CD 14+ monocytes characterized by cell surface expression of low levels of HLA-DR, CD86, CD16 and CD14. Flow cytometric analysis can be used to confirm the expression of these antigens. Monocytes can be isolated from any animal, including human, and from many sources including peripheral blood, cord blood, tonsils, spleens, bone marrow. CD14+ and other monocytes can be produced from stem cells, such as CD34+ cells, that are found in the previously mentioned sources. Production of monocytes from these stem cells requires the addition of factors such as GM-CSF.

When isolated from a subject, monocytes can be partially enriched by depletion of contaminating cells by methods known in the art, for example, by binding of antibodies that recognize these unwanted cells and removal of marked cells using magnetic micro-beads conjugated with antibodies that bind to the cell-bound antibody. Alternatively, CD14+ monocytes can be directly isolated with anti-CD14+ antibodies conjugated or bound to magnetic micro-beads. The direct isolation method was greatly improved by first depleting a small but significant amount of CD3+ lymphocytes from the monocyte cell source. CD3+ cells were removed with anti-CD3 antibodies and magnetic micro-beads as described in the Materials and Methods below. Other methods known to a person with ordinary skill in the art can be used to remove CD3+ cells and the efficiency of separation of cells can be monitored by measuring the purity of CD14+ monocytes in the sample.

When blood is used as a source, monocytes must first be separated from red blood cells, platelets, granulocytes and from other mononuclear cells such as lymphocytes and natural killer cells. Any conventional method for obtaining blood which maintains the viability of peripheral blood mononuclear cells can be used. Cell separation techniques for isolating PMBCs from blood are known to a person in the art. Such methods include standard gradient centrifugation with Ficoll-Hypaque, or Percoll, hypotonR, or direct lysis of red blood cells. CD14+ monocytes are then isolated from other mononuclear cells by methods discussed above such as positive selection using reagents which recognize and bind to a surface antigen specific for these cells such as CD14. For example, beads coated with mAb that recognizes CD14 are mixed with the mononuclear cells and the cells bound to the beads are removed. When magnetic beads are used, the adsorbed cells can be separated by a magnetic force. For immunobeads, the separation is done by gravity. Preferably, undesirable cells, i.e. any cell which competes and masks the differentiation of CD14+ monocytes, are removed or killed.

Monocytes obtained are cultured to form a primary culture on an appropriate substrate in a culture medium. This medium may be supplemented with autologous or nonautologous serum, or serum-free culture supplements. The appropriate substrate may be any tissue culture compatible surface. Preferably, the substrate is commercial plastic treated for use in tissue culture. Surfaces treated with a substance, for example poly-L-lysine may be used as long as they do not interfere with the proper differentiation of the monocytes to dendritic cells. Cells may also be maintained in suspension cultures in viscous gels, or

attached to suspended or fixed carrier substrates (Warren et al., 1995, Stem Cells 13, 167-174) Cells are preferably plated at a cell density which is adequate to maintain culture viability, i.e. the cells should not be over-crowded or the effect of IL-15 may be diminished or cells may die. An initial cell density of about 10 cels per cm^2 is a good starting point. At this dose, the surface is not fully covered by cells, but there are no big spaces (2-3 cm in diameter) either.

The growth medium for the cells should allow for the survival and proliferation of the monocytes. Any growth medium typically used to culture cells may be used according to the method of the invention. Preferred media include RPMI 1640, MEM, EMEM, or other complete media that is sufficient for support of primary cultures of mononuclear cells. Complete media, supplemented with human serum is preferred. However, serum-substitutes added to complete media is also preferred. Serum-free medium supplemented with growth factors is also suitable for culturing the monocytes. Cells may be selected or adapted to grow in other serums and at other concentrations of serum. Cells from human tissue may also be cultured in medium supplemented with fetal calf serum rather than human serum. Medias may contain antibiotics to minimize bacteria infection of the cultures. Penicillin, streptomycin or gentamicin or combinations containing them are preferred. The medium, or a portion of the medium, in which the cells are cultured should be periodically replenished to provide fresh nutrients.

IL15 cytokine has suprisingly been found to promote differentiation in vitro of monocytes into dendritic cells. Monocytes are cultured in the presence of IL-15 at a concentration sufficient to

promote differentiation of monocytes into dendritic cells. This can be determined by adding increasing amounts of cytokine to monocyte cultures and measuring the increase in HLA-DR expression as an indicator of DC transformation. Preferably, the cells are cultured in the presence of between about 1 and about 1000 ng/ml of IL-15, more preferably, about 10 to about 500 ng/ml, most preferably, about 20 to about 200 ng/ml.

IL-15 can be isolated from natural source, produced using recombinant DNA techniques or prepared by chemical synthesis. As used herein, IL-15 includes IL-15 produced by any method and from any species. "IL-15" is defined herein as any bioactive analog, fragment or derivative of the naturally occurring (native) IL-15. Such fragments or derivative forms of IL-15 should also promote the differentiation in culture of monocytes to dendritic cells. In addition, IL-15 peptides having biologic activity can be identified by their ability to bind IL-15 receptors on appropriate cell types.

It may be desirable to include other factors in the culture medium such as TNF-α, bacterial lipopolysaccharides, interferons α, β, γ, IL-18, cholera toxin, CpG-motif containing oligonucleotides, etc. which would be desirable for increasing the yield of dendritic cells, for inhibiting proliferation of possible cell contaminants.

Various techniques may be used to identify the cells present in the cultures. These techniques may include analysis of morphology, detecting cell type specific antigens with monoclonal antibodies, identifying proliferating cells using tritiated thymidine autoradiography, and demonstrating dendritic cell homing.

The dendritic cells besides being identified by their stellate shape may also be identified by detecting their expression of specific antigens using monoclonal antibodies. Among specific monoclonal antibodies suitable for identifying mature dendritic cells are: 1) those which bind to the MHC class I antigen (anti-MHC class I W6/32, Barnstabe et al. 1978 Cell 14, 9-20; 2) those which bind to MHC class II antigen (L243 anti-MHC class II [ATCC # HB55]; 3) CD80 (clone MAB104 Immunotech) CD86 (clone 2331 (FUN-1) Pharmingen). Those of skill in the art will recognize that other antibodies may be made and characterized which are suitable for identifying mature dendritic cells from different animals and sources.

Another index of dendritic cell maturity is the ability of mature dendritic cells to stimulate the antigen-specific proliferation of T cells. The ability of dendritic cells to migrate to lymph nodes, i.e., dendritic cell homing, or in vitro chemotaxis, are other indeces of dendritic cell maturation which may be used to assess the maturity of the cells in culture.

By being able to prepare dendritic cells in large numbers according to the method of this invention, other previously unexplored areas of dendritic function may now be determined. Specifically, growing dendritic cells will facilitate molecular and clinical studies on the mechanism of action of these APCs, including their capacities to capture and retain antigens in an immunogenic form and act as adjuvants for the generation of immunity in vivo.

Dendritic cells serve directly as APCs in situ, because the T cells that are primed are restricted to recognize only antigens presented by the particular

MHC class of the immunizing dendritic cells rather than antigens in an immunogenic form in situ. These observations, when coupled with data that dendritic cells are efficient at capturing protein antigens in an immunogenic form in situ, allow these APCs to be considered "nature's adjuvant". This invention therefore enables the utilization of dendritic cells by disclosing methods and compositions suitable for providing sufficient quantities of dendritic cells in order to take advantage of their unique antigen presenting capabilities in clinical and therapeutic practices. Antigens with which dendritic cells can be administered include but are not limited to microbial, tumor, and viral antigens.

Among the preferred embodiments of the invention, is a method for using dendritic cells to internalize particulates during their differentiation. When the particles are organisms which cause disease, such as mycobacteria, antigens associated with the dendritic cells are presented in a potent manner to T cells in vitro and in situ.

Foreign and autoantigens are processed by the dendritic cells of the invention to retain their immunogenic form. The immunogenic form of the antigen implies processing the antigen through fragmentation to produce a form of the antigen that can be recognized by and stimulate T cells. Preferably, such foreign or autoantigens are proteins which are processed into peptides by the dendritic cells. The relevant peptides which are produced by the dendritic cells may be extracted and purified for use as immunogens.

Peptides processed by the dendritic cells may also be used as toleragens to induce tolerance to the proteins processed by the dendritic cells of dendritic

cell precursors. Preferably when used as toleragens, the processed peptides are presented on dendritic cells which have been treated to reduce their capacity to provoke an immune response as by inhibiting their accessory function by blocking accessory molecules such as B7 present on the dendritic cells.

The antigen-exposed dendritic cells of the invention are produced by exposing antigen, in vitro, to the dendritic cells prepared according to the present invention. The immature DC rapidly take up and concentrate antigens, while the mature DC have a reduced capacity to do so. Preferably, the antigen is introduced into the monocyte cultures before transformation into DC has occured. Dendritic cells are plated in culture dishes and exposed to antigen in a sufficient amount and for a sufficient period of time to allow the antigen to bind to the dendritic cells. The amount and time necessary to achieve binding of the antigen to the dendritic cells may be determined by immunoassay or binding assay. Other methods known to those of skill in the art may be used to detect the presence of antigen on the dendritic cells following their exposure to antigen. The types of particles which may be internalized include bacteria, virus, mycobacteria, or other infectious agents capable of causing disease or soluble polypeptides and other macromolecules. Accordingly, any antigenic particle which is internalized and processed by the dendritic cell of this invention is also suitable for making the various immunogens, toleragens, and vaccines described as part of this invention. Processing of antigen by dendritic cells includes the fragmentation of an antigen into antigen fragments or modified antigens which are then presented.

In another embodiment, the dendritic cells may be injected with a vector which allows for the expression of specific proteins by the dendritic cells. These proteins which are expressed by the dendritic cell may then be processed and presented on the cell surface on MHC I receptors. The antigen-presenting cells or the processed antigens themselves may then be used as immunogens to produce an immunogenic response to the proteins encoded by the vector.

Vectors may be prepared to include specific DNA sequences which code and express genes from proteins to which an immunogenic response is desired. Several vectors can be used to introduce the immunogen such as retroviral vectors used to infect the dendritic cells, plasmid DNA vectors encoding the antigen, or a modified virus vector such as the alphavirus replicon. The use of these vectors is known to those skilled in the art and is described in Richard C. Mulligan, "Gene Transfer and Gene Therapy: Principle, Prospects and Perspective" in Etiology of Human Disease at the DNA Level. Chapter 12. J. Linsten and A. Peterson, eds. Rover Press, 1991 which is incorporated herein by reference.

The present invention provides a simple method for obtaining dendritic cells in sufficient quantities to be used to treat or immunize animals or humans with dendritic cells which have been activated with antigens. In addition, dendritic cells may be obtained in sufficient quantities to be useful as reagents to modify antigens in a manner to make the antigens more effective as T cell dependent antigens.

To use antigen-exposed dendritic cells as a therapeutic or immunogen the antigen-exposed dendritic cells are injected by any method which elicits an immune response into a syngeneic animal or human.

Preferably, dendritic cells are injected back into the same animal or human from whom the source monocytes were obtained. The injection site may be subcutaneous, intraperitoneal, intramuscular, intradermal, or intravenous. The number of antigen-exposed dendritic cells reinjected back into the animal or human in need of treatment may vary depending on the condition of patient, the antigen and size of the individual. A key feature in the function of dendritic cells in situ is the capacity to migrate or home to the T-dependent regions of lymphoid tissues, where the dendritic cells would be in an optimal position to select the requisite antigen-reactive T cells from the pool of recirculating quiescent lymphocytes and thereby initiate the T-dependent response.

According to the preferred method of stimulating an immune response in an individual, monocytes are isolated from an individual. It may be possible to increase the production of monocytes in the individual before isolating the cells so as to result in a larger number of DC. Monocytes may also be cryopreserved and thawed at a later date for later use. After isolation, the cells are cultured and exposed to IL-15 according to methods of the present inventions described above. The antigen is then introduced to the cells preferably at the start of culture. After sufficient time for the dendritic cells to process and present the antigen on their surfaces, the cell-antigen complexes are put back into the individual in sufficient quantity to evoke an immune response, somewhere in the range of 10^5 - 10^8, or more of less depending on the condition of the cells, the condition of the patient and the measured response to the therapy.

Antigen-exposed dendritic cells and dendritic cell modified antigens may both be used to elicit an immune response against an antigen. The activated dendritic cells or modified antigens may be used as vaccines to prevent future infection or may be used to activate the immune system to treat ongoing disease. The activated dendritic cells or modified antigens may be formulated for use as vaccine or pharmaceutical compositions with suitable carriers such as physiological saline or other injectable liquids. The vaccines or pharmaceutical compositions comprising the modified antigens or the antigen-exposed dendritic cells of the invention would be administered in therapeutically effective amounts sufficient to elicit an immune response. Preferably, between about 1 to 100 ug of modified antigen, or its equivalent when bound to dendritic cells, should be administered per dose.

Alternatively, T cells may be collected from the individual and exposed to the activated, antigen-presenting dendritic cells in vitro to stimulate antigen-specific T cells, which are then administered to the individual.

The activated antigen-presenting dendritic cells, or the dendritic cells can be used as a vaccine adjuvant and can be administered prior to, concurrently with or subsequent to antigen administration. The dendritic cells can be administered to the individual prior to, concurrently with or subsequent to administration of cytokines that modulate an immune reponse, for example interleukins 1, 2, 3, 4, 5, 7, 10, 12, 15, and 18, colony stimulating factors such as GM-CSF, or other cytokines such as TNF-alpha or interferon α, β, γ. Biologically

active derivatives of these cytokines and combinantions thereof will also be useful.

IL-15 can be administered directly to an individual to activate or cause the differentiation of cells that express the IL-15 receptor for the purpose of increasing the activity of dendritic cells. Alternatively, cells can be activated with IL-15 outside of the body, for the purpose of infusing these dendritic cells back into the individual or for obtaining substances such as processed peptides or cellular factors that can be used for indirect or direct immunotherapy for the same or another individual.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

The following materials and method were used in the examples below.

MATERIALS AND METHODS

The recombinant cytokines GM-CSF and IL-4 were obtained from Immunex (Seattle, WA), TNF-α from Genzyme Corporation (Cambridge, MA) and pooled human AB serum sera was were obtained from Pel-Freez (Brown Deer, WI). Mouse anti-human CD14 and CD3 mAbs conjugated with magnetic beads was were purchased from Milteneyi Biotech Inc., Auburn, CA. The FITC-conjugated mAbs Leu M3 (anti-CD14), Leu HLA DR (anti-DR), IL-2R (anti-CD25), anti-CD4, Leu 11a (anti-CD16), and Ig isotype control antibodies were purchased from Becton Dickinson (San Jose, CA). Anti-CD86, anti-CD11c, anti HLA-ABC, anti-GM-SCF were purchased from Pharmingen (San Diego, CA), anti-CD40 and anti-CD80

from Immunotech (Marseille, France), anti-CD1a antibody (cortical thymocytes) were obtained from Dako, (Carpinteria, CA). Anti-IL-15 was purchased from PeproTech, Inc (New Jersey, USA). Ficoll-Hypaque was purchased from Pharmacia, Uppsala, Sweden. The [methyl-3H] thymidine was purchased from Amersham Life Sciences (Arlington Heights, IL).

Cell separation and dendritic cell cultures

T lymphocytes (CD3+) are a major mononuclear cell population that often contaminates CD14+ selected monocytes. To obtain highly pure CD14+ cells from perepheral blood mononuclear cells (PBMC), our direct isolation method was greatly improved by first depleting CD3+ lymphocytes. CD3+ cells were removed with anti-CD3 antibodies, such as OKT3 (American Type Culture Collection or Miltenyi Biotech, Inc. Auburn, CA) that were attached to paramagnetic micro-beads. Peripheral blood mononuclear cells were obtained from normal healthy volunteers. Mononuclear cells were separated from blood by standard gradient centrifugation with Ficoll-Hypaque (Pharmacia). Mononuclear cells were harvested from the interface from cell medium and density gradient medium, washed twice, and purified mononuclear cells were suspended (10^7 cells/80 µl) in cold PBS supplemented with 2mM EDTA and 0.5% bovine serum albumin (Fraction V, Sigma Chemical Co., St. Louis, MO). Paramagnetic beads coated with anti-CD3 antibody were mixed with the mononuclear cells (20 µl per 10^7 cells). The CD3-labeled cells were incubated for 15 min (4°C), washed and passed through a type RS or VS iron-fiber column placed within a strong magnetic field (Miltenyi Biotech, Inc.). CD3+ cells bound magnetically to the column were separated. The flow-through containing

the unbound cells passed through the column were collected. The flow-through contains the monocytes further depleted from CD3+ cells by passing over a new column a second time to remove residual CD3-labeled cells. The efficiency of cell separation was monitored by flow cytometry using fluorescently-labeled, anti-CD3 antibody. Following T-cell depletion, CD14+ monocytes were then isolated from other mononuclear cells by positive selection using immuno-magnetic beads. Briefly, purified mononuclear cells were suspended (10^7 cells/80 ul) in cold PBS supplemented with 2mM EDTA and 0.5% bovine serum albumin (Fraction V, Sigma Chemical Co., St. Louis, MO). Paramagnetic beads coated with anti-CD14 mAb (Miltenyi Biotech Inc., Auburn, CA) were mixed with the mononuclear cells (20 µl per 10^7 cells). The CD14-labeled cells were incubated for 15 min (4°C), washed and passed through a type RS or VS iron-fiber column placed within a strong magnetic field (VarioMACS, Miltenyi Biotech, Inc.). The column containing the CD14+ cells was removed from the magnetic field and placed on a new collection tube. CD14+ monocytes bound to the column were mechanically eluted by pushing buffer through the column with a plunger. For purified T cells, mononuclear cells were centrifuged through discontinuous Percoll (Pharmacia) gradients (25 to 60%) and T cells (purity 95-98%) were obtained from the high density (45 to 60%) Percoll fraction as previously described elsewhere (Ortaldo et al., 1991). The isolated CD14+ monocytes did not express either IL-2Ra (CD25) or CD1a (data not shown). CD14+ monocytes were cultured (37°C, 6% CO_2) in RPMI-1640, supplemented with 5% human AB serum. For generation of control DC, cultures were also supplemented with 800 U/ml GM-CSF and 500 U/ml IL-4.

For experiments involving addition of cytokines, optimal doses were determined (data not shown), and based on these results, 100 ng/ml of TNF-α and 100 ng/ml of IL-15 were used for all studies.

FACS analysis

Before and after culture, cells were labeled with different fluorescence labeled-mAbs as described in reagents and analyzed by FACS. CD14+ monocytes cultured either with a combination of GM-CSF, IL-4 plus TNF-α or IL-15 alone were harvested, incubated with an anti-Fc receptor mAb (Miltenyi) blocking reagents to block Fc receptor binding sites, then incubated (45 min, 40°C) with different FITC-labeled or PE-labeled mAbs, or control isotype-matched mAbs. Unbound antibody was removed by washing the cells with media (40°C). After washing twice, cells were fixed with 1% paraformaldehyde prior to FACS analysis (Beckton Dickinson) and the cell-associated immunofluorescence was measured by flow cytometry (FACSort, Becton Dickinson).

Primary T-cell response to dendritic cells

CD14+ cells were cultured with either a combination of GM-CSF, IL-4 for 6 days followed by TNF-α treatment for 24 h or only IL-15 for 7 days in RPMI plus 5% human AB serum were harvested, irradiated (2000 Rad), and used as stimulator cells. T cells (1-3 x 10^5/well) were cultured (6 d, 370°C, 5% CO_2, humidified air) with irradiated dendritic cells as stimulators (1-10 x10^4/well) in 96-well, round bottom tissue culture plates (Costar, Cambridge, MA) with irradiated dendritic cells as stimulators (1-10 x10^4/well) in RPMI media containing 5% human AB serum. T-cell proliferation was measured in triplicate after

6 d of culture by incubating (12 h) cultures with 1μCi [^{3}H] thymidine/well (12 h) after 6 d of culture, harvesting the cells onto top count micro plate unifilters (Packward, Meriden, USA), and measuring radioactivity in a liquid scintillation Microplate Scintilation and Luminescence counter (Packward Instrument Company, Meriden, USA).

Measurement of transcriptional activation of chemokine genes by RT-PCR

Total cellular RNA was extracted from 1-2 x10^6 cells using TRI REAGENT (Molecular Research Center, Inc. Cincinnati, OH), according to the manufacturer's protocol. The concentration of purified RNA was estimated spectrophometrically by measuring the absorbance at 260nm. . Single-strand cDNA was synthesized from total RNA using GeneAmp RNA PCR kit containing AMV reverse transcriptase MuLVRT (Perkin Elmer, CA). Briefly, a 20 μl reaction mixture contained 250 ng of RNA template, 500 ng oligo-dT primer, 5mM $MgCl_2$, Tris buffer, 1mM dNTPs, 2.5 Units rRNAsin, and 15 units AMV reverse transcriptase. The reaction mixture was incubated for 30 min (42°C). Relative levels of chemokine mRNA were measured by PCR. The assay consisted of 2 μl of cDNA in a final volume of 50 μl that contained 800 mM Tris-HCl pH 8.9, 200 mM $(NH_4)_2SO_4$, 50 mM $MgCl_2$, 0.2 mM dNTPs, (Promega, Madison, WI), 0.2 μM of each primer and 1.5 Units AmpliTaq DNA Polymerase (Perkin-Elmer, Norwalk, CT). Primers used for PCR were: hMIP-1(: 5'-CGAGCCCACATTCCGTCACC-3' (SEQ ID NO:1) and5'-CGCATGTTCCCAAGGCTCAGG-3' (SEQ ID NO:2), amplifying a 309-bp product; hRANTES: 5'-CCCCGTGCCCACATCAAGGAGT-3' (SEQ ID NO:3) and 5'-TCAAGGAGCGGGTGGGGTAGGA-3' (SEQ ID NO:4), amplifying a 257-bp product; hPARC: 5'-

AGTTTCCAAGCCCCAGCTCACTCT-3' (SEQ ID NO:5) and 5'-TGGGGGCTGGTTTCAGAATAGTCA-3' (SEQ ID NO:6), amplifying a 208-bp product; hTARC: 5'-CCTCCTCCTGGGGGCTTCTCTG-3' (SEQ ID NO:7) and 5'-GACTTAATCTGGGCCCTTTGTGC-3' (SEQ ID NO:8 amplifying a 445-bp product; hELC: 5'-CACCCTCCATGGCCCTGCTACT-3' (SEQ ID NO:9) and 5'-TAACTGCTGCGGCGCTTCATCT-3' (SEQ ID NO:10) amplifying a 304-bp product; β-actin: 5'-ACACTGTGCCCATCTACGAGGGG-3' (SEQ ID NO:11) and 5'-ATGATGGAGTTGAAGGTAGTTTCGTGGAT-3' (SEQ ID NO:12), amplifying a 340-bp product. cDNA were amplified by PCR using following conditions: 60 s at 95°C, 3 min at 55°C and 2 min at 72°C for a total of 30 cycles. PCR products were resolved on a 1.5% agarose gel containing ethidium bromide.

Chemokine assays:

Chemokines MIP-1α, MIP-1β, MCP-1, RANTES, and IL-8 were measured by enzyme immunoassay (R&D SYSTEMS, Minneapolis, USA), using the manufacturer's instructions. Briefly, 100 µl of culture supernatant or control (standard) were added to 96-well microtiter plates pre-coated with mAb to MIP-1α, MIP-1β, MCP-1, RANTES, and IL-8 and incubated for 30 min at room temp (RT). The wells were washed and an enzyme-linked polyclonal antibody specific for MIP-1α, MIP-1β, MCP-1, RANTES, or IL-8 were added (100 µl) to detect bound cytokine. Following a brief incubation (30 min, RT), the wells were washed to remove any unbound antibody reagent, a substrate solution is added to the wells and incubated (20 min, RT). The color development was stopped and the intensity of the color was measured at the absorbance 450 nm. A standard curve was used to estimate the experimental concentration of chemokines.

EXAMPLE 1

IL-15 treated CD14+ monocytes acquire characteristic DC morphology in culture.

CD14+ monocytes when cultured in the presence of GM-CSF plus IL-4 followed by stimulation with TNF-α developed as a mature DC with long dendritic processes (Fig 1B) as also reported by others (Sallusto et al 1994, supra; Chapuis et al., 1997, supra; Zhou et al, 1996, supra). Thus it is evident that monocytes require growth factors such as GM-CSF plus IL-4 and inflammatory stimuli like TNF-α or LPS for maturation to DC. We examined whether IL-15 had any direct effect in the differentiation of CD14+ monocytes to mature DC. We observed that IL-15 directly induced differentiation of CD14+ monocytes to a large morphologically distinct population like mature DC. These cells had typical pronounced dendritic morphology, cytoplasmic protrusions, processes and veils (Fig.1C). This change in morphology of CD14+ monocytes indicates that monocytes responded to exogenous IL-15 and acquire dendritic features that are quite distinct from monocytes prior to culture (Fig. 1A). This preliminary observation led us to investigate further the hypothesis that IL-15 may transform monocytes to mature immuno-stimulatory DC.

EXAMPLE 2

Increased Surface expression of HLA-DR and CD86 molecules on mature DC induced by IL-15

Since there are no human DC-specific markers, cell surface expression of HLA-DR and co-stimulatory molecules such as CD86 antigens are considered diagnostic. IL-15 treated CD14+ monocytes were examined at different time points in culture for their surface HLA-DR and CD86 expression by flow cytometry.

Results shown in Fig. 2A and 2B indicate that IL-15 treated monocytes followed a distinct kinetics and expressed significantly higher levels of HLA-DR and CD86 when compared to untreated monocytes. These results indicate that IL-15 directly induced transformation of CD14+ monocytes to mature DC that is accompanied by strong HLA-DR and CD86 expression.

EXAMPLE 3

Phenotypic changes of CD14+ monocytes upon culture with a mixture of GM-CSF, IL-4 and TNF-α or IL-15 alone

Concomitant with the observed DC-like morphological changes and HLA-DR expression, significant cell surface molecular alterations appeared upon cultivation of CD14+ monocytes with IL-15. IL-15 induced mature DC expressed equivalent levels of cell surface antigens (co-stimulatory molecules, CD86, CD80 and CD40) compared to DC that were cultured with GM-CSF plus IL-4 and TNF-α (Fig. 3A and 3B). It was also noticed that IL-15 treated DC did not express IL-2Ra (CD25), CD1a and become CD14 negative (data not shown) further suggesting that mature DC derived from CD14+ monocytes upon exogenous IL-15 treatment were from myeloid origin.

EXAMPLE 4

Conversion of CD14+ monocytes to mature dendritic cells by IL-15 is independent of the GM-CSF, IL-4 and TNF-α driven pathway

GM-CSF appears to be a key factor for both murine and human DC development. It stimulates the growth and differentiation of pluripotential progenitors into DC both from myelomonocytic progeny as well as nonmyeloid lineages. Since monocytes upon culture with IL-15 distinctly up regulated the surface

expression of HLA-DR and CD86, next we examined whether the response of monocytes to IL-15 is independent of GM-CSF. For this we added different concentrations of neutralizing anti-GM-CSF antibody mAb (1ug/ml, 10ug/ml and 100 ug/ml) to the culture of monocytes that contained IL-15. Our results indicate that anti-GMCSF antibody did not block the expression of HLA-DR or CD86 molecules (Fig. 4A and 4B). In contrast, presence of anti-IL-15 antibody in culture significantly inhibited the development of mature DC phenotype and reduced the surface expression of HLA-DR (data not shown). This result suggests that the effect of IL-15 on monocytes is specific and independent of GM-CSF driven maturation pathway. Further, when we examined the release of IL-15 culture supernatants of GM-CSF/IL-4 activated monocytes, no detectable levels of IL-15 were observed (data not shown). Thus, our results indicate that the effect of IL-15 on monocytes does not require maturation stimuli like TNF-α or LPS.

EXAMPLE 6

IL-15 induced mature DC stimulate a strong response from T cells of unrelated donors

The best definition of DC relies on their functional properties. Potent induction of a primary, T-cell response is a measure of DC function. Therefore, we compared the allo-stimulatory capacity of mature DC that were generated by culturing CD14+ monocytes in the presence of IL-15 alone or GM-CSF, IL-4 plus TNF-α. Mature DC were cultured separately with freshly isolated highly purified T-cells to measure the stimulatory activity of mature DC. Results shown in Fig. 5 indicate that T-cell proliferation induced by DC that were matured by IL-15

treatment was equivalent to DC that were generated by treatment with a mixture of GM-CSF, IL-4 plus TNF-α. Thus, DC generated by IL-15 treatment carry out APC activity similar to that of conventional DC.

EXAMPLE 7

Chemokine production by mature DC

DC have been shown to both produce and respond to chemokines impacting their ability to function as antigen-bearing professional APCs that come in contact rapidly with large numbers of naive T-cells. DC physiology and functional maturation in generating strong immuno-stimulatory response is tightly linked with the induction of receptors and release of chemokines. To confirm that CD14+ monocytes when cultured with IL-15 generated mature DC that possessed distinct patterns of chemokine expression, we examined the transcriptional activation of multiple chemokine genes and analyzed the release of chemokines into culture supernatants. Results shown in Fig 6 indicate that IL-15 activated mature DC expressed multiple chemokine genes and released significant amounts of these chemokines into culture supernatants (Table 1). In contrast, monocytes prior to culture did not exhibit activation of these genes (Fig. 6). The CC family of inflammatory chemokines that includes macrophage inflammatory protein MIP-1α, macrophage chemotactic protein MCP-1, RANTES, are produced significantly more by IL-15 induced DC (Table 1) compared to DC that were induced by the mixture of GM-CSF, IL-4 and TNF-α (Table 1). These results are in agreement with previous reports where IL-15 activated human monocytes were shown to produce MCP-1 and IL-8 (Badolato et al., 1997, Blood 90, 2804). The constitutive chemokines such as pulmonary and

activation regulated chemokine (PARC), thymus and activation-regulated chemokine (TARC) which are DC-specific, were also up regulated in mature DC when cultured with IL-15. These results indicate that IL-15 induced mature DC expressed distinct chemokine genes that may provide DC with the capacity to regulate chemotaxis and hence the recruitment of other cells of the immune system.

Table 1. Production of Cchemokines secreted following induction ofby mature dentritic cells.DC maturation upon GM-CSF/IL-4/TNF-α or IL-15 treatment

	pg/ml				
	RANTES	MIP-1α	MIP-1α	IL-8	MCP-1
Media[a]	<13.1	<31.2	<31.2	<62.5	<31.2
No cytokine[b]	249	700	>4000	24286	7789
GM-CSF/IL-4 TNF-α	575	993	>4000	8724	3089
IL-15	3438	>2000	>4000	25858	8278

[a] Negative control (without cells and cytokine)

[b] Cells in serum containing medium without cytokine

Discussion

Our results demonstrate that IL-15 directly induces the transformation of CD14+ monocytes to mature DC. These IL-15 induced mature dendritic cells were similar to classic myeloid DC that were generated

with a combination of GM-CSF, IL-4 and TNF-α in morphology, surface phenotype, chemokine expression and induction of strong allo-response from T-cells. Addition of anti-GM-CSF antibody in culture did not inhibit development of mature dendritic cell and HLA-DR, CD86 expression. In contrast, anti-IL-15 antibody inhibited the development of CD14+ monocytes to mature DC morphology and HLA-DR, CD86 expression. IL-15 induced dendritic cell maturation from CD14+ monocytes, did not require inflammatory stimuli to support APC activity, and was independent of the GM-CSF-driven pathway of maturation. Taken together, these data support a distinct role for IL-15 in the recruitment and transformation of monocytes to mature DC.

Monocytes can be driven to mature immuno-stimulatory DC in ex vivo culture with multiple cytokine cocktails, growth factors and inflammatory stimuli like TNF-α, LPS etc (Cella et al., 1997). Culture of CD34+ hematopoietic progenitor cells with IL-15 treatment has recently been shown to induce their differentiation into phenotypically discrete populations of NK and DC (Bykovskaia et. al., 1999, supra). Thus, IL-15 can induce uncommitted progenitors into distinct immune regulatory cells (Bykovskaia et. al., 1999, supra; Waldmann and Tagaya 1996, supra; Ma et al. 2000, supra). However, the effect of IL-15 on APCs including monocytes in ex vivo culture or during infection is still unclear. The expression of IL-15 and IL-15Rα in virtually all tissues contrasts sharply with the highly restricted expression of IL-2 and IL-2R, and hints that IL-15 may have a broader role in stimulating immune responses (Carson et al., 1994, J. Exp. Med. 180, 1395). In fact, it is reported that IL-15 contributes in enhancing antigen-specific

immunity against infectious pathogens (Khan and Kasper, 1996, J. Immunol. 157, 2103; Nishimura et al., 1996, J. Immunol. 156, 663; Jullien et al., 1997, J. Immunol. 158, 800), however, the effect of IL-15 on recruitment and activation of professional antigen presenting cells in these studies has not been demonstrated. Our ex vivo study indicates IL-15 directly induces maturation of CD14+ monocytes to DC that are known for inducing both primary and antigen-specific immune response.

It is known that IL-15 is expressed at late stage of dendritic cell culture by a combination of GM-CSF, IL-4, TNF-α or interferon type 1(IFN), GM-CSF or GM-CSF, IL-4 and LPS treatment but not by GM-CSF, IL-4 (Blauvelt et. al., 1996, J. Invest. Dermatol. 106, 1047; Santini et al. 2000, J Exp. Med. 191, 1777). In this study, anti-GM-SCF antibody did not inhibit transformation of monocytes to DC whereas anti-IL-15 antibody inhibited transformation of monocytes to DC phenotype and significantly blocked HLA-DR and CD86 expression (data not shown). Thus, DC maturation from CD14+ monocytes by IL-15 is altogether a different signaling pathway. Therefore, it is reasonable to assume that activation of DC from CD14+ monocytes in response to IL-15 appears to be an important aspect for amplifying primary immune responses against infectious agents or vaccines. This assumption is strengthened by the fact that resting monocytes do not produce IL-15 whereas DC cultures produce detectable level of IL-15 protein (Jonuleit et al, 1997, J. Immunol. 158, 2610). Furthermore, release of IL-15 by primary targets or activated monocytes may act as a positive loop in generating professional APCs such as DC (Kuniyoshi et. al., 1999, Cell. Immunol. 193, 48). In this study, exogenous IL-15 alone induced the

maturation of CD14+ monocytes to DC with requisite functional characteristics further suggesting that IL-15 driven monocytes-to-DC conversion may be a mechanism for maintaining the antigen-specific adaptive immunity. This hypothesis is in agreement with in vivo observations that the availability of IL-15 would likely facilitate stimulation of the specific cellular immune responses (Maeurer et. al., 1999, J. Immunol. 50, 280; Khan and Casciotti 1999, J. Immunol. 163, 4503).

Chemokine production by mature DC has consequences not only for recruitment of other cell types but also for the function of the mature DC for homing from the inflammatory sites to the T and B cell areas of secondary lymphoid organs. Key aspects of DC maturation include the abundant production of inflammatory chemokines such as MIP-1α, MIP-1β, and RANTES produced by maturing activated DC and activation of other chemokine genes such as hELC, hTARC and hPARC are activated in mature DC (Sallusto et al., 1999, Eur. J. Immunol. 29, 1671; Greaves et al. 1997, J. Exp. Med. 186, 837). The production of chemokines by mature DC facilitates the recruitment of other mononuclear cells and granulocytes as well as homing of DC from inflammatory sites to the T- and B-cell areas of secondary lymphoid organs. Prior to IL-15 treatment, CD14+ monocytes did not show activation of these chemokine genes further suggesting that induction of maturation by IL-15 would allow DC to migrate to specific sites in order to activate effector cells. Unlike IL-2, which is preferentially secreted by T cells, IL-15 has much broader tissue distribution, i.e. expressed by non-lymphoid tissues, epithelial, fibroblasts cell lines, and activated monocytes/macrophages allow DC access to different

tissues for recruiting and stimulating effector cells. In summary, our report provides the first evidence that IL-15 serves directly in transforming CD14+ monocytes to mature DC that are considered to be the conductors in controlling immune responses.

What is claimed is:

1. A method for producing cultures of dendritic cells comprising:

(i) obtaining monocytes from a tissue source;

(ii) contacting said monocytes with a sufficient amount of interleukin-15 for a sufficient period of time to result in differentiation of monocytes into dendritic cells.

2. The method of claim 1, wherein said tissue source is human blood.

3. The method of claim 2, further comprising removing CD3+ mononuclear cells after step (i) and prior to step (ii).

4. A composition comprising dendritic cells produced by the method of claim 1.

5. A composition comprising dendritic cells produced by the method of claim 3.

6. The composition according to claim 4 wherein said dendritic cells have been exposed to an antigen rendering the dendritic cells antigen-exposed.

7. A method for providing to a subject immunity to an antigen comprising administering to a subject in need of such immunity an immunogenic amount of antigen-exposed dendritic cells wherein said antigen-exposed dendritic cells have been exposed to said antigen.

8. The method of claim 7 wherein said antigen is a peptide.

9. The method of claim 8 wherein said peptide is selected from the group consisting of: viral peptide, bacterial peptide, parasitic peptide, and cancer cell peptide.

10. The method of claim 7 wherein said dendritic cells were obtained from said subject.

11. A method for inducing a T cell-mediated immune response to an antigen in a subject, comprising:

(i) exposing dendritic cells produced by the method of claim 1 to said antigen such that they become antigen-exposed dendritic cells;

(ii) administering said antigen-exposed dendritic cells to said subject.

12. The method of claim 11 wherein said dendritic cells are obtained from monocytes obtained from said subject.

13. A method for stimulating an antigen-specific immune response comprising the steps of:

(i) obtaining monocytes from an individual;

(ii) obtaining dendritic cells by exposing, ex vivo, the cells of step (i) with IL-15 suitable for culturing dendritic cells;

(iii) exposing the dendritic cells of step (ii) with a gene encoding an antigen, so as to obtain antigen-expressing dendritic cells; and

(iv) administering an effective amount of the activated antigen-expressing dendritic cells of step

(iii) to the individual so as to stimulate the antigen-specific immune response in said individual.

14. A method for producing modified antigens comprising immunogenic fragments of a native antigen comprising exposing a culture of dendritic cells prepared by the method of claim 1 to said native antigen such that antigen is modified by said dendritic cells.

15. Modified antigens produced by the method of claim 14.

16. A method for inducing in an individual an immune response to a native antigen comprising administering to said individual an amount of modified antigen according to claim 15 effective for producing said immune response.

17. A method for making an autoantigen comprising exposing dendritic cells produced by the method of claim 1 to with a protein to which an individual has devloped an immune response and extracting the autoantigen.

18. An autoantigen produced by the method of claim 17.

19. A method for treating an individual with an autoimmune disease comprising administering to said individual a therapeutically effective amount of autoantigen according to claim 18 such that tolerance to said autoantigen is produced in said individual.

20. A method for activating T cells comprising presenting modified antigens according to claim 15 to T cells in vitro or in situ.

21. A vaccine against an antigen comprising modified antigens according to claim 15.

22. A vaccine against an antigen comprising antigen-exposed dendritic cells according to claim 6.

23. An adjuvant comprising antigen-exposed dendritic cells according to claim 6.

FIG. 1A

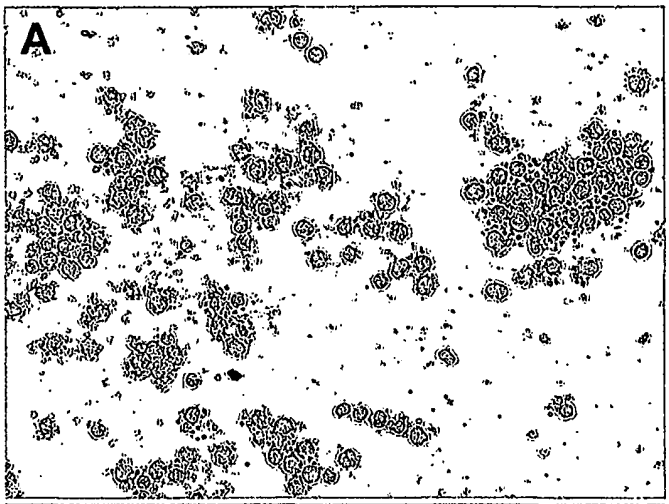


FIG. 1B

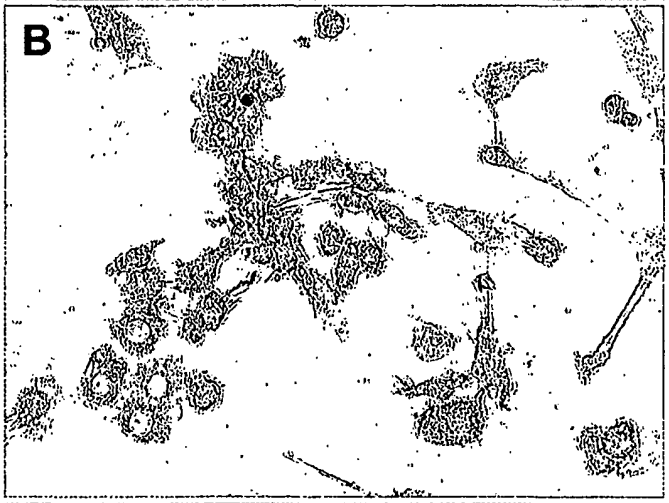


FIG. 1C

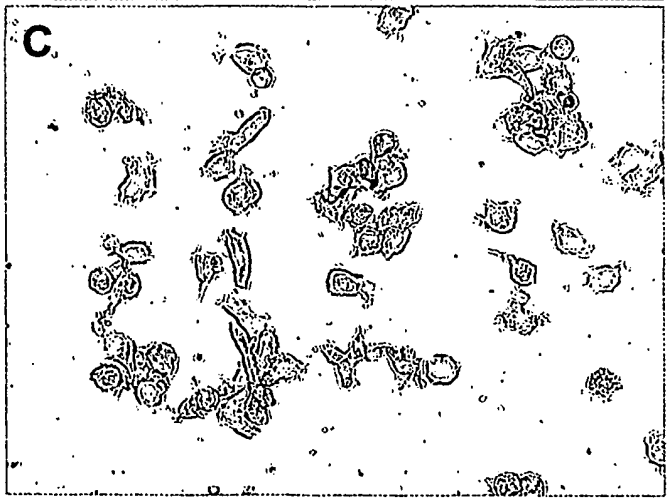


FIG. 2A

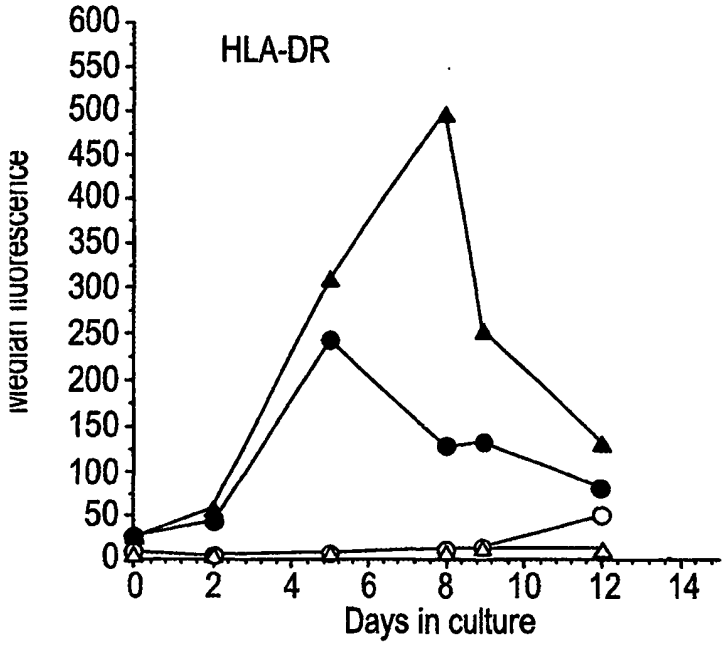

HLA-DR
Median fluorescence
600
550
500
450
400
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12 14
Days in culture

FIG. 2B

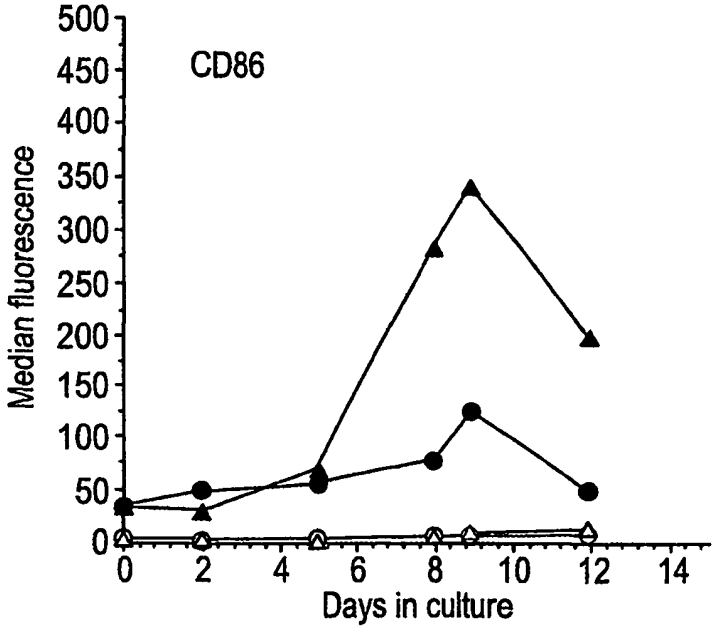

CD86
Median fluorescence
500
450
400
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12 14
Days in culture

FIG. 3A

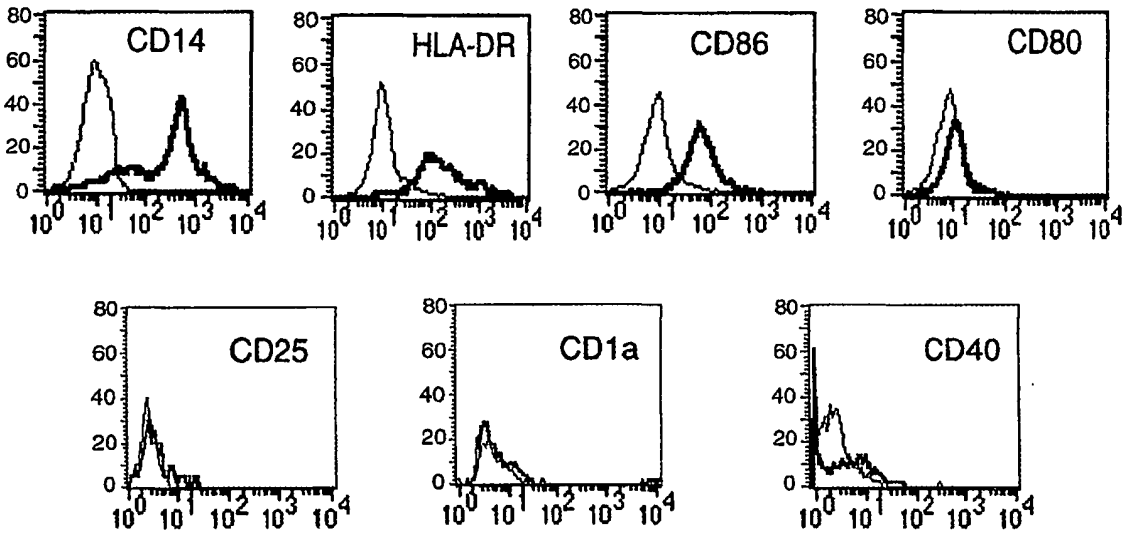


FIG. 3B

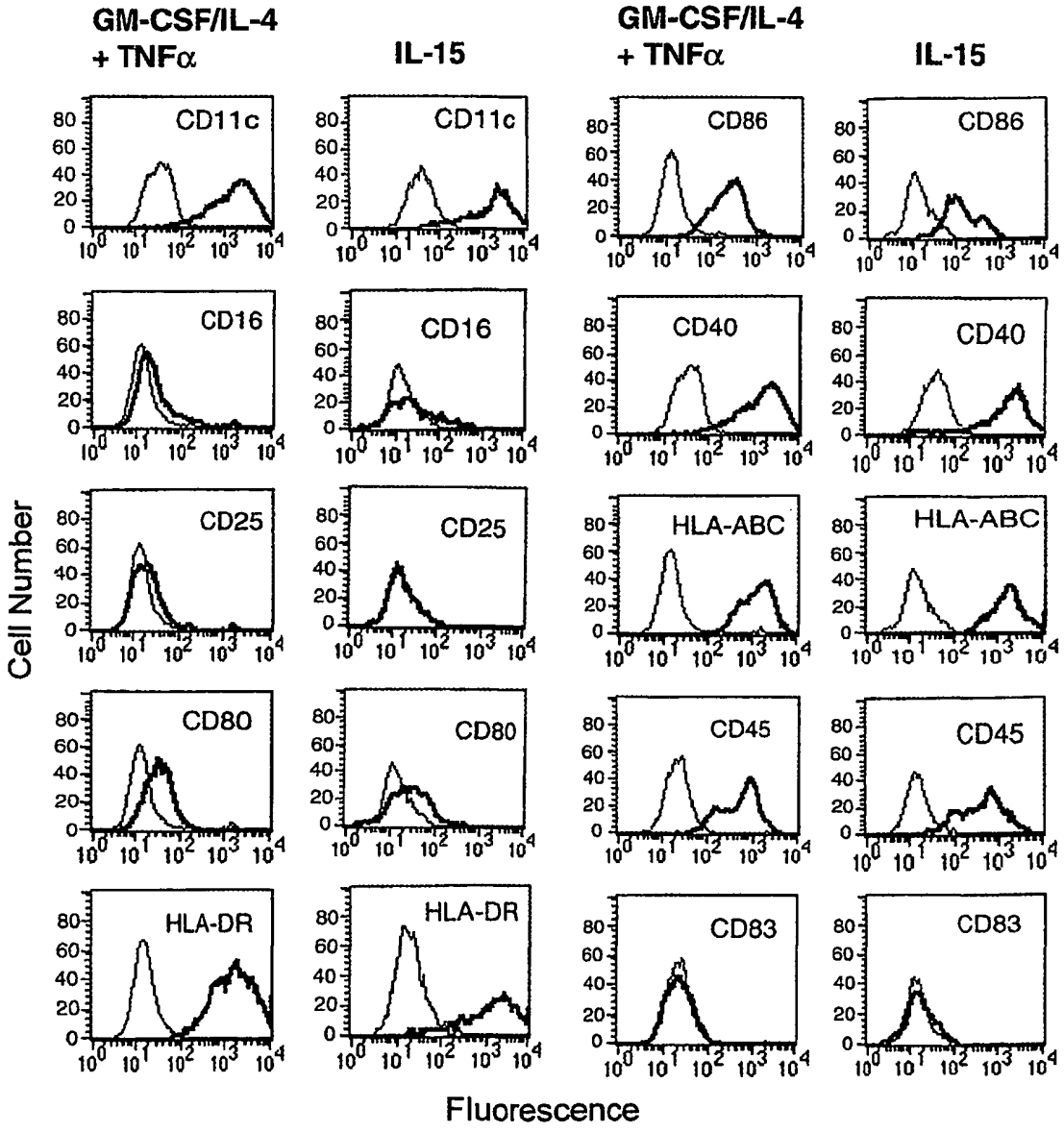

GM-CSF/IL-4 + TNFα
IL-15
GM-CSF/IL-4 + TNFα
IL-15
CD11c
CD11c
CD86
CD86
CD16
CD16
CD40
CD40
CD25
CD25
HLA-ABC
HLA-ABC
CD80
CD80
CD45
CD45
HLA-DR
HLA-DR
CD83
CD83
Cell Number
Fluorescence

FIG. 4A

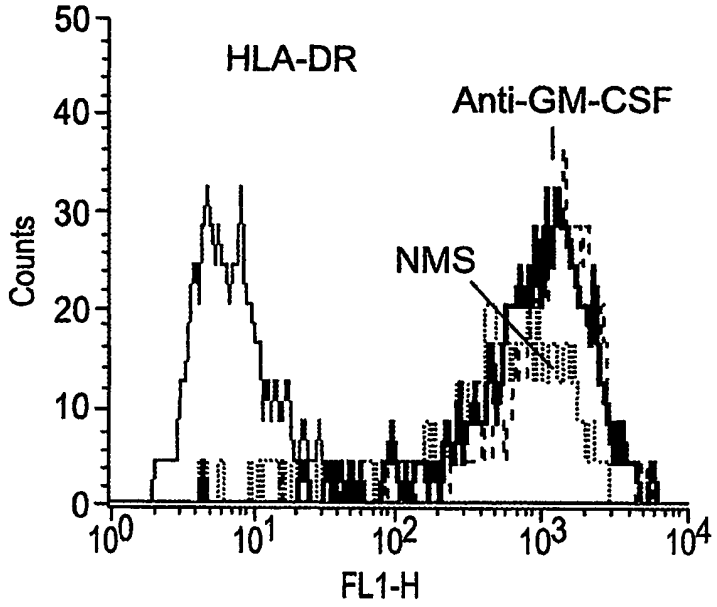

HLA-DR
Anti-GM-CSF
NMS
Counts
FL1-H
0
10
20
30
40
50
10^0
10^1
10^2
10^3
10^4

FIG. 4B

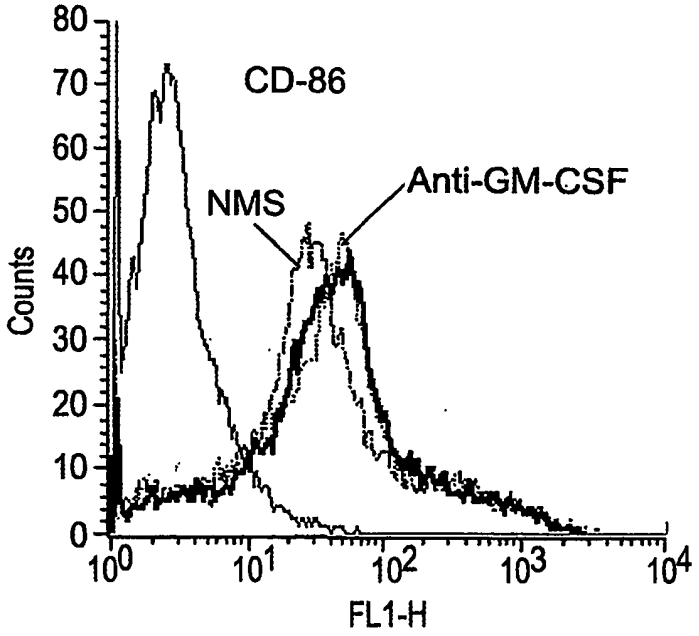

CD-86
NMS
Anti-GM-CSF
Counts
FL1-H
0
10
20
30
40
50
60
70
80
10^0
10^1
10^2
10^3
10^4

FIG. 5

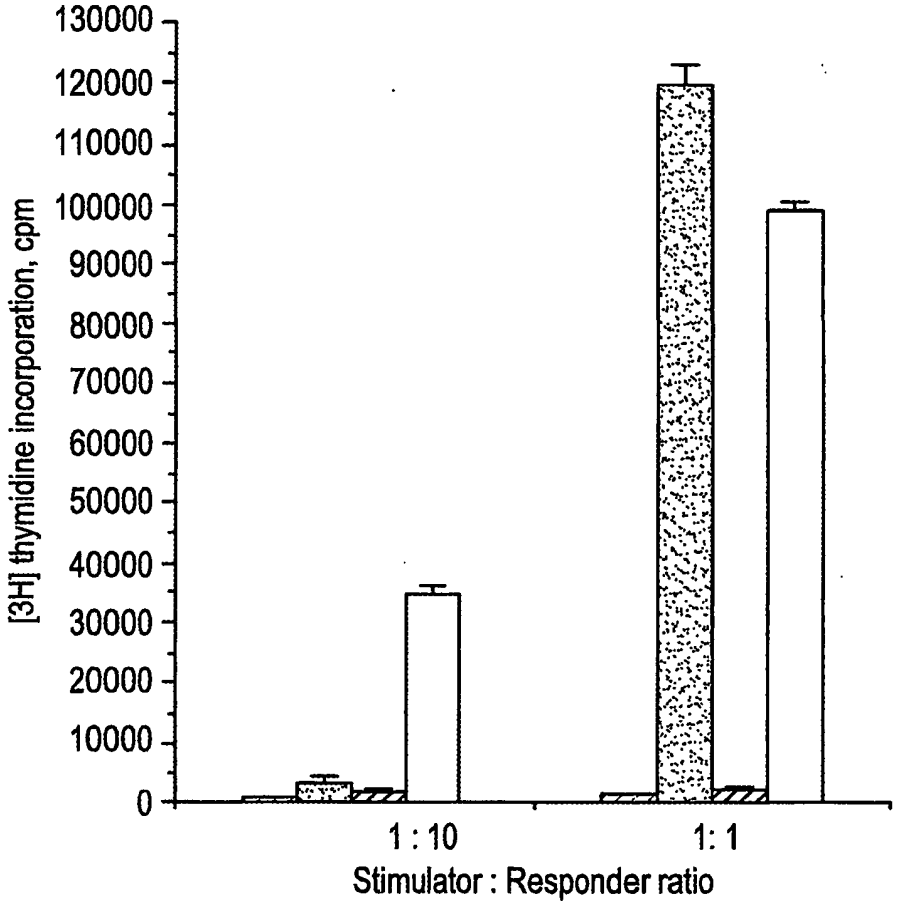
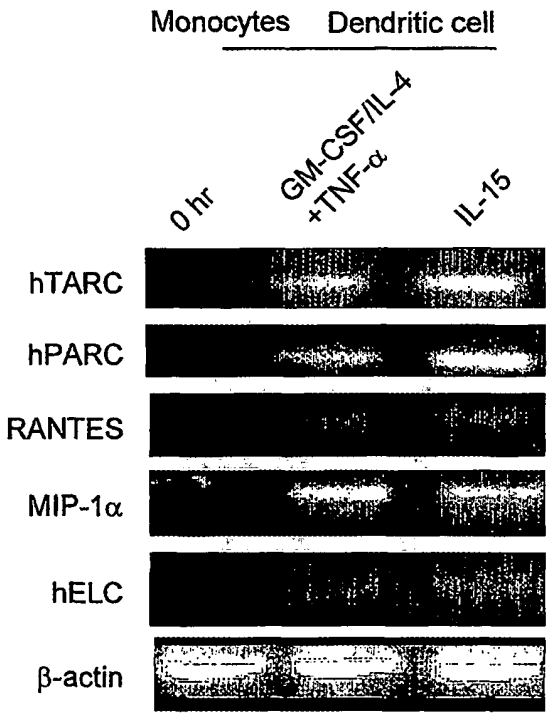

130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
[3H] thymidine incorporation, cpm
1 : 10
1: 1
Stimulator : Responder ratio

FIG. 6


Monocytes
Dendritic cell
0 hr
GM-CSF/IL-4 +TNF-α
IL-15
hTARC
hPARC
RANTES
MIP-1α
hELC
β-actin

SEQUENCE LISTING

SEQ ID NO:1
hMIP-1 primer: 5'-CGAGCCCACATTCCGTCACC-3'

SEQ ID NO:2 5'-CGCATGTTCCCAAGGCTCAGG-3'

SEQ ID NO:3
hRANTES primer: 5'-CCCCGTGCCCACATCAAGGAGT-3'

SEQ ID NO:4
5'-TCAAGGAGCGGGTGGGGTAGGA-3'

SEQ ID NO:5
hPARC: 5'-AGTTTCCAAGCCCCAGCTCACTCT-3' () and

SEQ ID NO:6
5'-TGGGGGCTGGTTTCAGAATAGTCA-3'

SEQ ID NO:7
hTARC: 5'-CCTCCTCCTGGGGGCTTCTCTG-3'

SEQ ID NO:8
5'-GACTTAATCTGGGCCCTTTGTGC-3'

SEQ ID NO:9
hELC: 5'-CACCCTCCATGGCCCTGCTACT-3'

SEQ ID NO:10
5'-TAACTGCTGCGGCGCTTCATCT-3'

SEQ ID NO:11
β-actin: 5'-ACACTGTGCCCATCTACGAGGGG-3'

SEQ ID NO:12
ATGATGGAGTTGAAGGTAGTTTCGTGGAT-3'

Int al Application No
PCT/US 00/31465

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06 C12N5/08 A61K35/14 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAIKH K U ET AL: "IL-15 DIRECTLY INDUCES MATURATION OF CD14+ MONOCYTES TO DENDRITIC CELLS" FASEB JOURNAL,FED. OF AMERICAN SOC. FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD,US, vol. 14, 20 April 2000 (2000-04-20), page A1050 XP000996576 ISSN: 0892-6638 abstract --- -/--	1-23

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2001	28/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nichogiannopoulou, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ial Application No
PCT/US 00/31465

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BYKOVSKAIA S ET AL: "THE GENERATION OF HUMAN DENDRITIC AND NK CELLS FROM HEMOPOIETIC PROGENITORS INDUCED BY INTERLEUKIN-15" JOURNAL OF LEUKOCYTE BIOLOGY,FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL,US, vol. 66, October 1999 (1999-10), pages 659-666, XP000996602 ISSN: 0741-5400 the whole document	1-23
A	L -J ZHOU ET AL: "CD14+ BLOOD MONOCYTES CAN DIFFERENTIATE INTO FUNCTIONALLY MATURE CD83+ DENDRITIC CELLS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, no. 93, 1 March 1996 (1996-03-01), pages 2588-2592, XP002075945 ISSN: 0027-8424 the whole document	1-23
A	MA AVERIL ET AL: "The pleiotropic functions of interleukin 15: Not so interleukin 2-like after all." JOURNAL OF EXPERIMENTAL MEDICINE, vol. 191, no. 5, 6 March 2000 (2000-03-06), pages 753-755, XP002169910 ISSN: 0022-1007 the whole document	1